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Phosphorylation of STAT1 Serine 727 Enhances Platinum Resistance in Uterine Serous Carcinoma(Abstract_要旨)

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京都大学	博士（医学）	氏 名	曾 翔
論文題目	Phosphorylation of STAT1 Serine 727 Enhances Platinum Resistance in Uterine Serous Carcinoma （子宮体部漿液性癌において、STAT1 のセリン 727 リン酸化はプラチナ抵抗性に関わる）		
（論文内容の要旨）			
<p>Uterine serous carcinoma (USC) is a highly aggressive histological subtype of endometrial cancers and associated with high chemo-resistant features and poor prognostic outcomes. The previous study showed that STAT1 is highly expressed in USC and acts as a key molecule that is positively correlated with tumor progression, but it remains unclear whether STAT1 is relevant to the malicious chemo-refractory nature of USC. The principal aim of this study was to clarify whether targeting STAT1 can be a potential strategy to overcome platinum resistance in USC to improve clinical outcomes.</p> <p>In the present study, first of all, STAT1 expression status was confirmed by IHC in USC patient samples (n=40) from Kyoto university hospital. Tumor with high STAT1 expression was associated with significantly reduced overall survival and progression-free survival compared to those with low STAT1 expression. Suppressing STAT1 expression by shRNA in Spac1L cells led to increase cisplatin-mediated apoptosis. This observation was further confirmed by comparing STAT1 expression and cisplatin sensitivity in three USC cell lines: Spac1L, ARK1 and ARK2. STAT1 was highly expressed in ARK2 and Spac1L cells, and higher STAT1-expressing cells were more resistant to cisplatin. Together, these data suggested that STAT1 might play an important role in cisplatin chemo-sensitivity in USC cells.</p> <p>Next, to explore the mechanism involved in high STAT1 cisplatin resistance characteristic, DNA damage, DNA repair, cellular accumulation of cisplatin, and cisplatin inactivation were comprehensively investigated. As the result, DNA damage marker, Gamma-H2AX was significantly increased in shSTAT1 cells under cisplatin treatment. However, no remarkable differences were found between shSTAT1 cells and mock cells in five main DNA repair pathways. Other than DNA damage and repair, significant increase of intracellular cisplatin accumulation was observed in shSTAT1 cells. These finding was also confirmed by three USC cells: intracellular cisplatin accumulation was enhanced (86%) in lowest STAT1 expressing ARK1 cells, while this enhancement was weaker (10%) in Spac1L cells, and almost not observed (0.56%) in highest STAT1 expressing ARK2 cells. These results indicated that sensitivity of shSTAT1 cells to cisplatin was correlated with increasing DNA damage by enhancing cisplatin intracellular accumulation, but not engaged in DNA repair process.</p> <p>Next, STAT1 transduction on chemoresistance was investigated. As STAT1 modulates its downstream pathways via phosphorylation at serine727 or tyrosine701, phosphorylation of STAT1 under cisplatin treatment was accessed. Cisplatin exposure induced nuclear phosphorylation of STAT1 on serine727, but not on tyrosine701. Moreover, induction of dominant-negative pSTAT1 serine mutant plasmid significantly increased cisplatin sensitivity by enhancing cisplatin-inducing apoptosis and cisplatin accumulation. Furthermore, cisplatin induced nuclear pSTAT1 serine phosphorylation was significantly inhibited by TBB, a serine-threonine kinase II, casein kinase2 (CK2) inhibitor. When TBB was added before cisplatin treatment on both Spac1L and ARK2 cells, increased apoptosis and enhancing intracellular cisplatin accumulation were observed. Given these findings, pretreatment with TBB for suppressing STAT1 serine phosphorylation could be a potential strategy to attenuate cisplatin resistance in cancer cells.</p> <p>Finally, <i>in vivo</i> experiments by inhibiting pSTAT1 serine with TBB were carried out. Tumor load was significantly reduced by combination therapy of TBB and cisplatin in both Spac1L and ARK2 xenograft models. Furthermore, the tumor growth of ARK2 pSTAT1 serine mutant bearing mice was significantly inhibited by cisplatin treatment compared to wildtype ARK2 bearing mice.</p> <p>These results of this study collectively suggested that pSTAT1 serine may play a key role in platinum resistance as well as tumor progression in USC. Thus, targeting the STAT1 pathway via CK2 inhibitor can be a novel method for attenuating the chemo-refractory nature of USC.</p>			

(論文審査の結果の要旨)			
<p>本論文では、子宮体癌の中でも難治性である子宮体部漿液性癌（USC）において、転写因子 STAT1 が新規治療標的となるかを明らかにすることを目的として研究を行った。</p> <p>臨床検体を用いた検討により STAT1 を高発現する USC は生命予後が不良であった。そこで、子宮体癌細胞株を用いた検討により STAT1 発現の高い USC 細胞（STAT1^{high}-USC）ではシスプラチンを投与してもアポトーシスが誘導されにくいことが明らかとなった。さらに STAT1^{high}-USC は、シスプラチン添加により、STAT1 のチロシン残基ではなくセリン残基がリン酸化し (pSTAT1-Ser727)、さらに pSTAT1-Ser727 が核内に集積してシスプラチンと DNA の結合を阻害することによって、プラチナ抵抗性を示すことが明らかとなった。また、セリンリン酸化阻害剤である TBB を前投与すると、STAT1^{high}-USC に対するシスプラチンのアポトーシス誘導効果が上がることを <i>in vitro</i> および <i>in vivo</i> にて示した。</p> <p>これらの結果から、STAT1 はセリン残基のリン酸化を介してプラチナ抵抗性を示すことが明らかとなり、STAT1 のリン酸化機序が USC に対する治療効果改善に繋がる可能性を示した。</p> <p>以上の研究は、プラチナ製剤抵抗性に関する新機序の解明に貢献し今後のがん治療の発展に寄与するところが多い。</p>			
<p>以上の研究は、プラチナ製剤抵抗性に関する新機序の解明に貢献し今後のがん治療の発展に寄与するところが多い。</p>			
<p>したがって、本論文は博士（医学）の学位論文として価値あるものと認める。</p>			
<p>なお、本学位授与申請者は、令和１年９月１９日実施の論文内容とそれに関連した試問を受け、合格と認められたものである</p>			
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